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# EVALUATING THE EFFECTS OF HIGH-DOSE MELATONIN ON MENTAL AND SOMATIC STATUS OF NORMAL SUBJECTS




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19. ABSTRACT: Pulmonary and central nervous system oxygen toxicity are mission-limiting, health-endangering consequences of breathing high partial pressures of oxygen. Melatonin, a hormone produced by the pineal gland, is a powerful antioxidant that can be safely given at high dosages to human subjects. We tested in ten human subjects the mental and somatic effects of 300 mg of pharmaceutical-grade melatonin administered orally. The dosage was divided into two boluses given four hours apart. The subjects maintained a normal daytime working schedule after dosing. Each subject completed an ANAM 2000 neuropsychological performance battery administered one hour after each dose; some subjects repeated ANAM testing two hours after the first dose. The scores from the tests were compared to predosing baseline scores. This dosage of melatonin did not trigger any adverse reactions and caused no significant degradation of ANAM scores. Matching to Sample scores increased significantly. Both objective measures and subjective impressions varied widely, with no clear pattern overall. Only half the subjects reported an increase in drowsiness following the administration of melatonin.

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## INTRODUCTION

This study is part of an effort to assess the prophylactic efficacy of melatonin, a known antioxidant, in combating the deleterious effects of high partial pressures of oxygen.[1] This initial effort was designed to confirm the safety of administering high doses of pure melatonin to human subjects.

Pulmonary and central nervous system (CNS) oxygen toxicity represents a mission-limiting, health-endangering consequence of breathing high partial pressures of oxygen ($PO_2$) during diving operations. Because of $O_2$ toxicity, U.S. military combat swimmers are limited to 240 minutes of "on-oxygen" time every 24 hours.[2] In principle, pharmaceutical-based detoxification could improve diver safety, prolong mission durations, and improve the tempo of sustained military operations.

Melatonin (*N*-acetyl-5-methoxytryptamine), a naturally occurring hormone and neurotransmitter produced by the brain's pineal gland, is a candidate for protecting the body against high $PO_2$ exposure because of its efficacy as an antioxidant, as evidenced by animal studies[3–5] and by its safety. A review by Reiter provides a comprehensive list of melatonin's various protective pathways.[6] Using a 10 mg/kg dose in rats, Pablos et al provided the earliest preclinical data on melatonin's antioxidant efficacy during hyperbaric oxygen exposures.[3] In that work, all indices of oxygen-induced damage were reduced to control levels or lower when melatonin was administered before a 90-minute exposure to 4.0 atmospheres absolute (atm abs) $O_2$. For a 70 kg human, the analogous prophylactic dosage would be 700 mg.

The Office of Dietary Supplements, National Institutes of Health, classifies melatonin as a dietary supplement. Therefore, no current dosage limitations exist. For over-the-counter melatonin used as a sleeping aid, the usual dosage is 1–3 mg.

Melatonin has no known toxic dose level in either humans or animals; a dose of 800 $mg \cdot kg^{-1}$ did not cause death in mice.[7] An equivalent dosage in a 70 kg man would be 56 grams.

Consequently, investigative studies of effects unrelated to sleep induction have used it in varying dosages. A study using melatonin in Alzheimer's patients cited four references in which patients took daily doses of 300 mg or more, in many cases for weeks or months, with no reported side effects.[8] One study reported no side effects or signs of toxicity among 32 women receiving 300 mg daily for four months;[9] another reported complete absence of side effects among all eight healthy male subjects following two 500 mg doses taken 30 min apart.[10]

On the other hand, substantially smaller doses have been reported to exert sedative effects. For example, healthy male volunteers in one study took daily doses of 10, 20, 40, or 80 mg of melatonin just before noon for five days.[11] When compared with control subjects who took placebos, test subjects who had taken melatonin were affected by all melatonin doses: melatonin significantly decreased the number of correct responses during auditory vigilance testing, increased the subjects' reaction times, and reduced their self-reported levels of "vigor." Moreover, melatonin also increased their self-reported levels of fatigue, confusion, and sleepiness.

This study sought to determine the side effects, if any, of a physically fit Navy diver population taking relatively large oral doses of melatonin during the daytime. A special focus of the study was on the neuropsychological status of the subjects as determined by a computerized assessment battery and components of the Halstead-Reitan Neuropsychological Test Battery (HRNB).[12]

The Automated Neuropsychological Assessment Metrics (ANAM) battery was created to assess an individual's intellectual functioning, from superior function to moderately impaired. It provides a strong measurement of sustained attention or concentration, spatial processing, mental flexibility, short- and long-term memory, working memory, fatigue, and level of arousal. All the tests assess a person's abilities to attend to detail and remain mentally alert, obviously important capabilities for military missions.

## METHODS

Ten physically fit subjects (2 female, 8 male) volunteering for the melatonin dosing trials[13] completed current physicals and signed consent forms approved by NEDU's Committee for the Protection of Human Subjects. Between 0700 and 0800 hrs in the morning, each subject ingested two 150 mg doses of chromatographically pure melatonin obtained from Helsinn Pharmaceuticals (Pazzallo, Switzerland). Each dose was dissolved in 0.5 to 1.0 mL ethanol and diluted in 8 oz (~240 mL) of orange juice. Doses were administered four hours apart on an empty stomach to subjects who, after waiting three to five minutes, then consumed a typical meal for that time of day.

One hour after each melatonin administration, we tested subjects' motor performance with grip strength, finger tapping, and Harvard Step Tests. Subjects were then scored on the ANAM, a computer-based neuropsychological testing system. The components of the ANAM 2000 battery included were reaction time, matching to sample, running memory, and mathematical processing tasks. Subjects manifesting performance decrements one hour after the first melatonin administration were retested one hour later.

Other neuropsychological assessments, such as grip strength and finger tapping rates for both hands, were also used. Grip strength was measured on a Jamar Hydraulic Hand Dynamometer, Standard Model, and lower body agility was tested with a Harvard Step Test. Test subjects were also asked to make notes of any unusual symptoms or sensations while they conducted their normal business throughout the day.

All subjects performed at least one ANAM session as a baseline within one week of the beginning of the study. They reported any unusual or unpleasant effects of melatonin on the questionnaire in Annex A.

### ANAM-M

The ANAM[14,15] is a computer-based standard clinical subset of the Office of Military Performance Assessment Technology (OMPAT) Tester's Workbench (TWB). ANAM is a set of TWB tests reconfigured for use in clinical neuropsychological evaluations. Many components of the ANAM were derived from the Unified Tri-service Committee

Performance Assessment Battery (UTC-PAB)[16] and the Walter Reed Performance Assessment Battery.[17] The ANAM purports to measure mental efficiency as well as accuracy.[15]

The ANAM test equipment consisted of Micron Transport Trek II laptop computers (Micron PC; Nampa, ID) with 366 MHz Pentium processors, a standard mouse, and the ANAM software.

The variables considered in the ANAM tests were mean reaction time (MRT), the average response latency in milliseconds for the duration of each test; accuracy (ACC), the percentage of correct responses for each test; and throughput (Thruput), a measure of the number of correct responses made each minute.

The data were compiled with the Statview feature of ANAM[13] and transferred to Microsoft Excel for storage and manipulation. The data were then transferred to Jandel's SigmaStat (Jandel Scientific; San Rafael, CA) and analyzed by a paired T-test if the raw data passed tests for normality. Data not passing the normality test were exposed to the nonparametric Signed Rank Test for analysis of significant differences.

The tests in the ANAM battery were selected for assessing sustained concentration and attention, mental flexibility, spatial processing, cognitive processing efficiency, arousal and fatigue levels, and working memory. Specifically, the ANAM-M consisted of the following subtests:

- Demographics form
- Stanford Sleepiness Scale (SLEEP; measures alertness/fatigue levels)
- Simple Reaction Time (SRT; measures basic psychomotor speed)
- Matching to Sample (M2S; measures delayed recall/longer-term memory)
- Running Memory Continuous Performance Task (CPT; measures working memory and executive functions)
- Mathematical Processing Task (MATH; measures computational speed and working memory)

These subtests and the functions they are purported to measure are discussed in more detail in Reeves et al.[15]

Brief summaries of the neuropsychological tests are given as follows:

SLEEP: This is a scale consisting of seven statements that describe how one feels with respect to alertness or sleepiness. This scale has been designed to provide a state and/or trait assessment of energy-fatigue levels. The measures from this scale include the score from the scale and the latency of response in milliseconds.

Table 1. An Introspective Measure of Sleepiness

| **Degree of Sleepiness** | **Scale Rating** |
|---|---|
| Feeling active, vital, alert, or wide awake | 1 |
| Functioning at high levels, but not at peak; able to concentrate | 2 |
| Awake, but relaxed; responsive, but not fully alert | 3 |
| Somewhat foggy, let down | 4 |
| Foggy; losing interest in remaining awake; slowed down | 5 |
| Sleepy, woozy, fighting sleep; prefer to lie down | 6 |
| No longer fighting sleep, sleep onset soon; having dreamlike thoughts | 7 |
| Asleep | X |

SRT: Simple Reaction Time serves two purposes. The first is to provide a measure of pure reaction time, and the second is to provide a means to parcel out the effects of motor or peripheral nerve conduction velocity times from actual cognitive processing time. This test presents a simple stimulus (e.g., * ) on the screen. The participant is instructed to press a specified response key each time the stimulus is presented. The on-screen characters are large and easily seen by older subjects. The resulting measures are MRT, which is response latency, and Thruput, which is a derived measure that purportedly measures mental efficiency.

M2S: Matching to Sample is a test in which the participant is required to match a block pattern from memory. A single 4 x 4 matrix (i.e., a checkerboard) is presented in the center of the screen as a sample stimulus. For each trial presentation of a matrix, the number of cells that are shaded varies at random. Following a specified time interval (for this study, five seconds), two comparison matrices are presented side by side. One of the comparison matrices matches the "sample" matrix, while the other comparison matrix differs in shading from the sample by one cell. The subject's task is to indicate, by pressing the appropriate response button, which matrix matches the sample matrix. This test measures spatial processing and visuospatial working memory. The resulting measures are MRT, ACC (a percentage of correct responses), and Thruput.

CPT: Running memory is a continuous letter or number comparison task. In the running-memory task, participants are asked to monitor a randomized sequence of

uppercase letters A through Z or numbers 0 through 9. The stimuli are presented one at a time in the center of the screen. Participants are asked to continuously monitor the letters and press a specified key or button if the character on the screen matches the letter that immediately preceded it. Subjects are requested to press a different response button or key if the character does not match the immediately preceding letter. CPT measures concentration (attention), working memory, and lapses in attention. The resulting measures are MRT, ACC, and Thruput.

MATH: During this task, arithmetic problems are presented in the middle of the screen. The task involves deducing an answer and then determining whether the answer is greater or less than the number 5. Each problem includes two mathematical operations (addition and/or subtraction) on sets of three single-digit numbers (e.g., 5 + 3 – 4 = ?). The subject is instructed to read and calculate from left to right and indicate whether the answer is greater or less than 5 by pressing one of two specified response buttons. The operators and numbers are selected at random, with the following restrictions: only the digits 1 through 9 are used; the correct answer may be any number (except 5) from 1 to 9; test problems with answers greater or less than 5 are equally probable; cumulative intermediate totals have a positive value; working left to right, the same digit cannot appear twice in the same problem unless it is preceded by the same operator on each occasion (e.g., +3 and +3 are acceptable, while +3 and -3 are not); and the sum of the absolute value of the digits in a problem must be greater than 5. This test purports to index basic computational skills, concentration, and working memory. The resulting measures are MRT, ACC, and Thruput.

Finger Tapping: The finger tapping test is a component of the HRNB used for assessing motor cortex functioning of the brain. Speeded motor performance is determined by the speed of finger tapping separately for the index finger of the right and left hand.

Grip Strength: In conjunction with finger tapping, grip strength — a cortical motor test measuring motor speed (finger tapping) and strength (grip strength) — is one of the most common measurements used in neuropsychological assessments. A hand dynamometer is used for this measurement, and as in finger tapping, the test is conducted with both the dominant and nondominant hands.

Step Test: A modified Harvard Step Test was used to assess lower body strength, mobility, and coordination. Subjects mounted and dismounted a set of steps as many times as possible during 60 seconds. Safety spotters flanked subjects as they performed the test. The steps were of the standard Harvard Step Test form: two steps, each 10 inches high, for a total vertical rise of 20 inches. When the subject placed his foot on the lower step, an infrared beam was broken and an infrared receiver sent a signal to a laptop computer that recorded the number of times the beam was broken.[18]

## RESULTS

Subject means are based on a sample size of 10 subjects for the following three test periods: baseline, one hour after the first dose, and one hour after the second dose. The means taken two hours after the first dose are based on the number of subjects

who participated, a number that depends on how many subjects had shown a degradation in their performances (Table 1) between the baseline and the test given one hour after the first dose. No more than four subjects took the tests two hours after the first dose.

Of all comparisons between pretest baselines and measurements made one hour after the first or second dose of melatonin, only two showed statistically significant differences. Compared to the baseline measurements, a statistically significant improvement was found in matching measured one hour after the second dose of melatonin ($p = 0.002$), as determined by the Wilcoxon Signed Rank Test.

Table 2. Subject Sample Size for Tests Given 2 Hours after the First Dose

| Test | Number of Subjects |
|---|---|
| Reaction Time | 4 |
| Running Memory | 4 |
| Math Processing | 4 |
| Matching | 4 |
| Harvard Step | 2 |
| Dominant Hand Grip Strength | 2 |
| Nondominant Hand Grip Strength | 2 |
| Dominant Finger Tapping | 2 |
| Nondominant Finger Tapping | 2 |

Simple Reaction Time

In Figure 1, the ten subjects' reaction times in milliseconds (ms) increased beyond 10% of their baselines, and the individual performances of two other subjects were between 10 and 15% of their baseline times. All three reaction times for only one subject decreased by more than 5% of his baselines. As for the other five subjects, their times remained within 6% of their baselines. After the ten subjects received doses of melatonin, there was no significant change in reaction times (Table 3).

In Figures 1 through 9 a bar over the baseline represents performance identical to the baseline; the absence of a bar in the graph represents missing data.

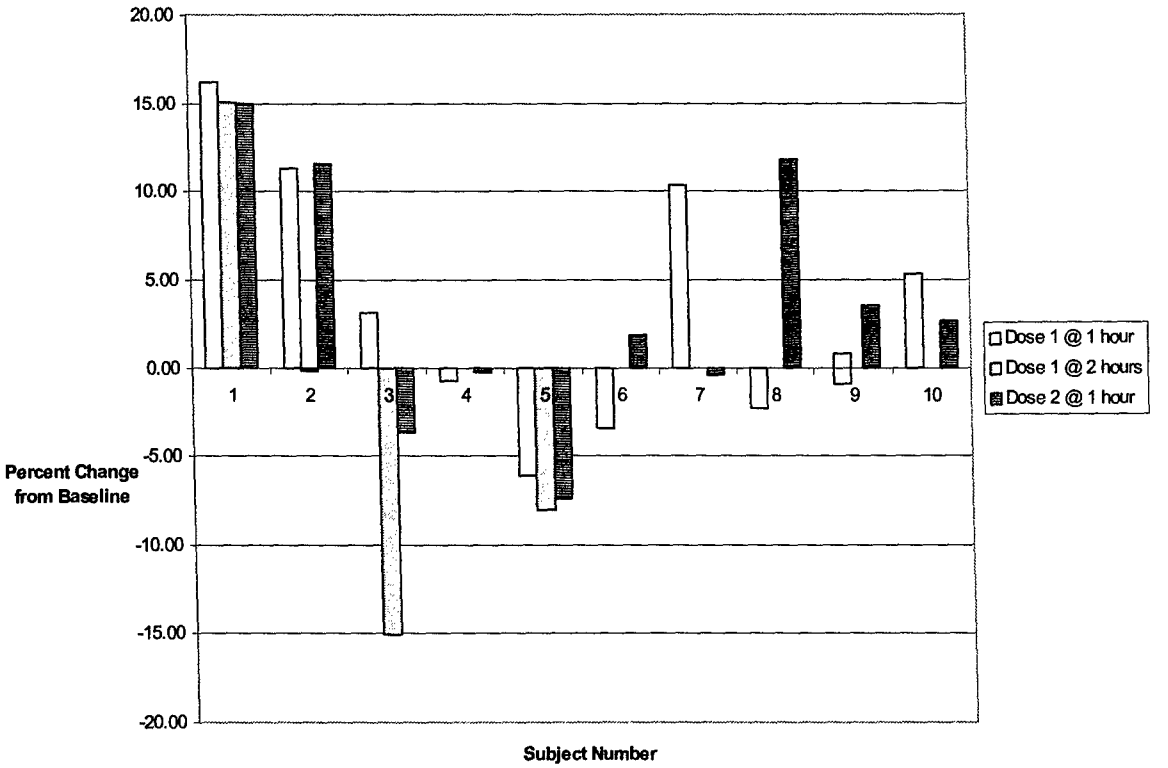


**Figure 1.** Percentage Change from Baseline Reaction Time. Values below 0 on the Y-axis represent an improvement in performance; values above 0 represent a decrement.

Table 3. Mean Reaction Times (ms)

| Measurement Point | Thruput mean ± SD | Reaction Time (ms) |
|---|---|---|
| Baseline (n = 10) | 220.1 ± 43.5 | 285.1 ± 109.4 |
| 1 hour after first dose (n = 10) | 227.7 ± 23.2 | 260.8 ± 30.6 |
| 2 hours after first dose (n = 4) | 236.5 ± 28.1 | -- |
| 1 hour after second dose (n = 10) | 229.4 ± 21.3 | 260.6 ± 26.2 |

Running Memory

Eight of ten subjects remained within 20% of their baseline Running Memory Thruput performances (Figure 2); only one subject lost more than 10% from his baseline. For two subjects, running memory scores improved between 60 and 100% of their baselines; nevertheless, the mean running memory scores did not differ significantly from the mean baseline score (Table 4).

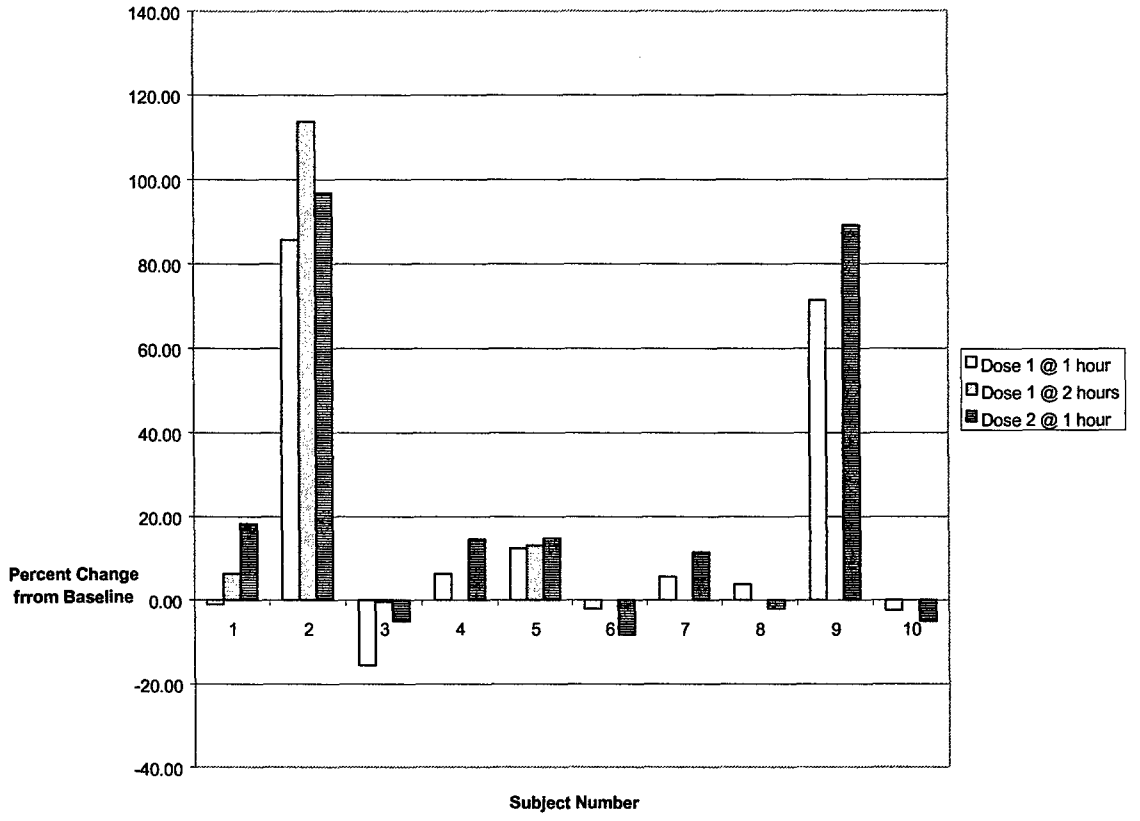


**Figure 2.** Percentage Change from Baseline Running Memory Thruput.

Table 4. Subject Means: Running Memory

| | Thruput mean ± SD | Reaction Time (ms) | Accuracy (% correct) |
|---|---|---|---|
| Baseline (n = 10) | 115.0 ± 33.9 | 524 ± 177 | 87.9 ± 20.4 |
| 1 hour after first dose (n = 10) | 124.7 ± 21.6 | 479 ± 84 | 97.0 ± 1.6 |
| 2 hours after first dose (n = 4) | 122.3 ± 21.4 | -- | -- |
| 1 hour after second dose (n = 10) | 129.8 ± 17.9 | 463 ± 64 | 97.8 ± 2.2 |

Math Processing

The math processing performances of two subjects declined between 20 and 40% from their baseline levels (Figure 3). However, overall there were no significant changes in math processing scores. The means of the ten subjects' math processing scores show little change from baseline to the three postdose tests (Table 5).

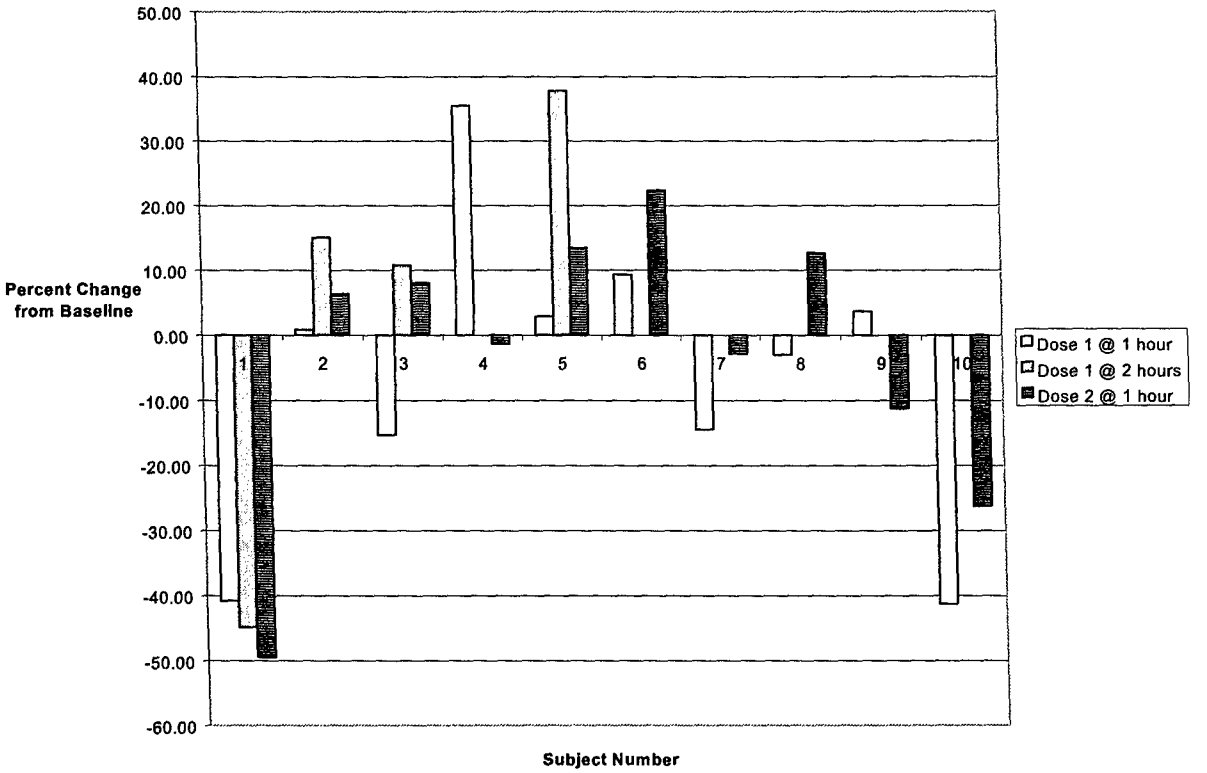


Figure 3. Percentage Change from Baseline in Math Processing Thruput.

Table 5. Subject Means: Math Processing (mean ± SD)

| | Thruput | Reaction Time (ms) | Accuracy (% correct) |
|---|---|---|---|
| Baseline (n = 10) | 35.1 ± 5.9 | 1773 ± 427 | 97.0 ± 4.2 |
| 1 hour after first dose (n = 10) | 32.3 ± 7.5 | 1844 ± 478 | 95.0 ± 6.2 |
| 2 hours after first dose (n = 4) | 33.4 ± 10.9 | -- | -- |
| 1 hour after second dose (n = 10) | 34.0 ± 8.8 | 1879 ± 734 | 97.5 ± 3.5 |

Matching to Sample

Eight of ten subjects' matching performances remained at or above baseline levels across all test trials (Figure 4). One hour after his first dose of melatonin, one subject's M2S performance declined 34% from his baseline; another subject's increased 195% from his baseline performance one hour after the second dose. The means of the subjects show increasing scores throughout the matching tests (Table 6).

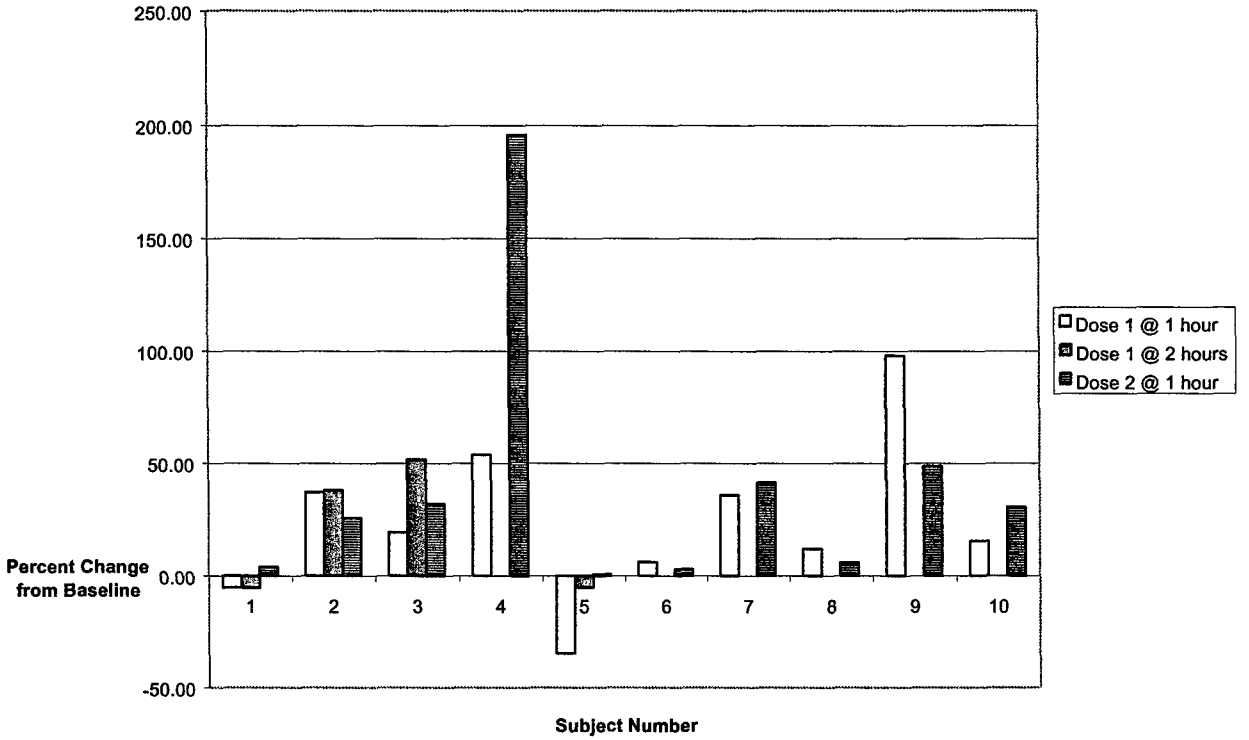


**Figure 4.** Percentage Change from Baseline in Matching.

Table 6. Subject Means: Matching

| | Thruput mean ± SD | Reaction Time mean ± SD (ms) | Accuracy (% correct) |
|---|---|---|---|
| Baseline (n = 10) | 40.0 ± 13.6 | 1444 ± 472 | 94 ± 11 |
| 1 hour after first dose (n = 10) | 46.8 ± 13.0 | 1295 ± 384 | 95 ± 7 |
| 2 hours after first dose (n = 2) | 43.8 ± 8.6 | -- | -- |
| 1 hour after second dose (n = 10) | 53.0 ± 20.8 | 1294 ± 416 | 98 ± 4 |

There was a trend toward increasing matching Thruput as both time and melatonin dose increased, a trend that reached statistical significance one hour after the second dose. The data obtained one hour after the second dose failed the normality test, and thus a

nonparameteric Wilcoxon Signed Rank Test was run, with p = 0.002 indicating a statistically significant difference.

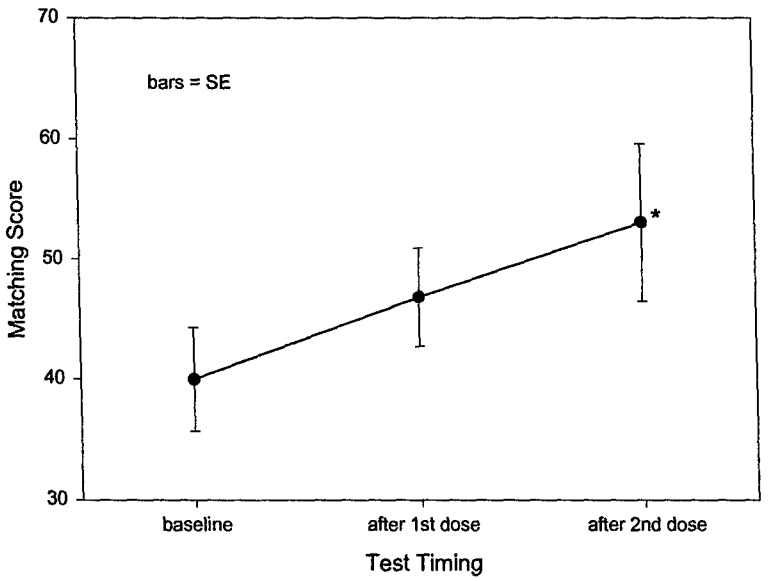


**Figure 5.** Scores on the matching portion of ANAM-M at baseline and after one and two doses of melatonin. An asterisk indicates a statistically significant difference from baseline.

Harvard Step Test

For nine of ten subjects, step test scores remained at their baselines or improved up to 25%; for only one subject did baseline performance decline 75 to 90% throughout the three trials (Figure 6). Performance means for all the subjects show little change from the baselines, a result suggesting that step test performance is unaffected by the melatonin (Table 7).

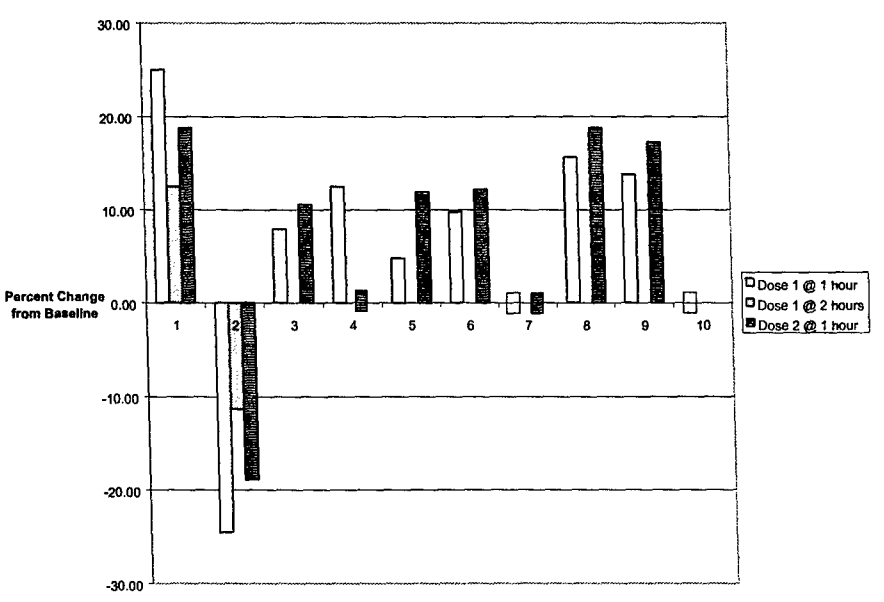


**Figure 6.** Percentage Change from Baseline in Harvard Step Test.

Table 7. Subject Means: Harvard Step Test

| | Averages mean ± SD |
|---|---|
| Baseline (n = 10) | 38.0 ± 7.4 |
| 1 hour after first dose (n = 10) | 39.7 ± 4.2 |
| 2 hours after first dose (n = 2) | 41.5 ± 7.8 |
| 1 hour after second dose (n = 10) | 34.6 ± 5.2 |

Dominant Hand Grip Strength

Dominant hand (DH) grip strength was unaffected by the melatonin dosing: all subjects' strength remained within 20% of their baseline performances (Figure 7). The reduction in strength two hours after the first dose (Table 8) was for two subjects who performed at the low end of the test group throughout the grip strength tests.

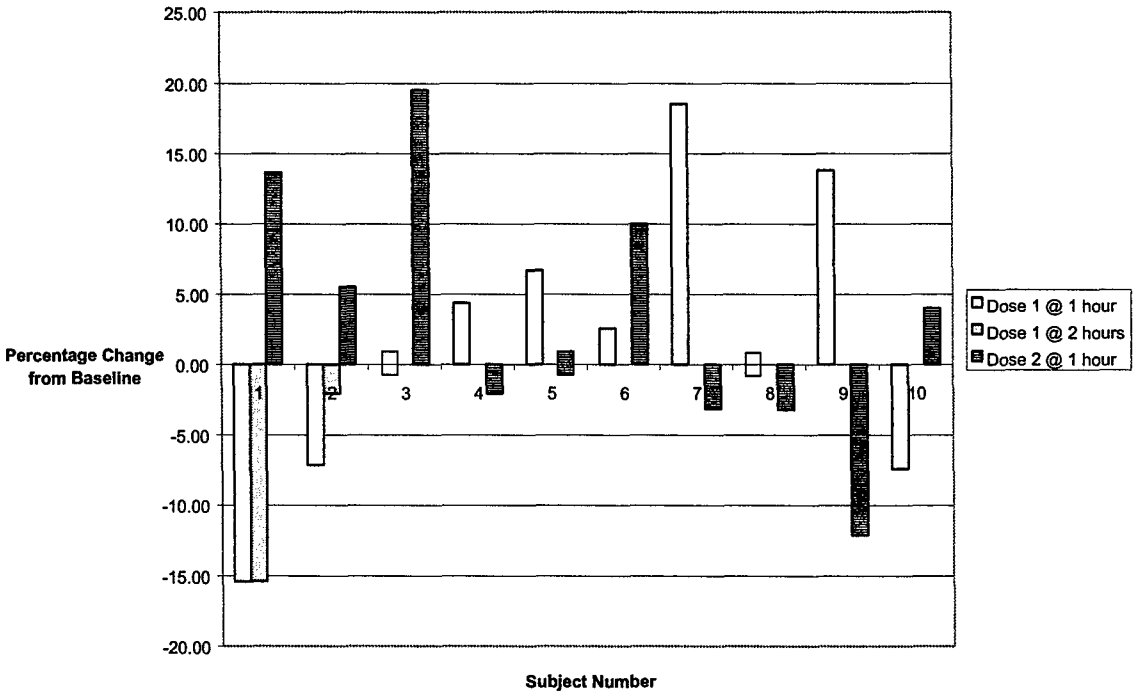


**Figure 7.** Percentage Change from Baseline in Dominant Hand Grip Strength.

Table 8. Subject Means: Dominant Hand Grip Strength

| | Averages mean ± SD |
|---|---|
| Baseline (n = 10) | 45.9 ± 11.9 |
| 1 hour after first dose (n = 10) | 47.1 ± 14.2 |
| 2 hours after first dose (n = 2) | 35.0 ± 18.4 |
| 1 hour after second dose (n = 10) | 47.7 ± 11.8 |

Nondominant Hand Grip Strength

Eight of ten subjects' nondominant (ND) hand grip strength remained at or above 90% of baseline across all test trials (Figure 8). Nine subjects remained within 25% of their baseline performances; one subject's performance diminished to around 70% of the baseline for two of the three tests. These results show that melatonin has no appreciable effect on grip strength. The means of the ten subjects demonstrate this, even though the mean value two hours after the first dose is low. That mean was low because, once again, the two subjects who were retested consistently scored low on all the grip strength tests (Table 9).

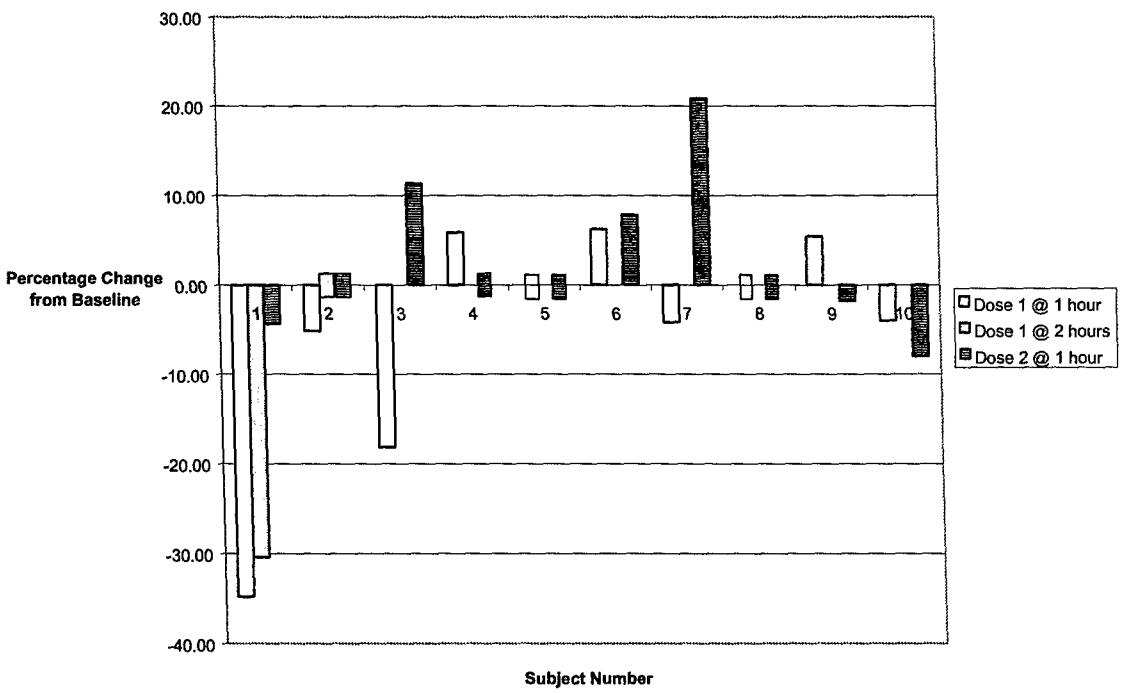


**Figure 8.** Percentage Change from Baseline in Nondominant Hand Grip Strength.

Table 9. Subject Means: Nondominant Hand Grip Strength

| | Averages mean ± SD |
|---|---|
| Baseline (n = 10) | 41.6 ± 13.0 |
| 1 hour after first dose (n = 10) | 40.1 ± 14.1 |
| 2 hours after first dose (n = 2) | 27.5 ± 16.3 |
| 1 hour after second dose (n = 10) | 42.8 ± 13.7 |

Dominant Hand Finger Tapping

Eight of ten subjects' DH finger tapping remained at or above 90% of baseline across all test trials. In only two instances did subjects fail to perform within 90 to 120% of their baselines (Figure 9). One subject's tapping rate was reduced to around 80%, and another dropped to 68% of baseline. The means show the same proximity to the baselines as do those of the grip tests, with one notable exception: the test performed two hours after the first dose (Table 10). Again, this drop in performance is for those two subjects whose baselines are among the lowest.

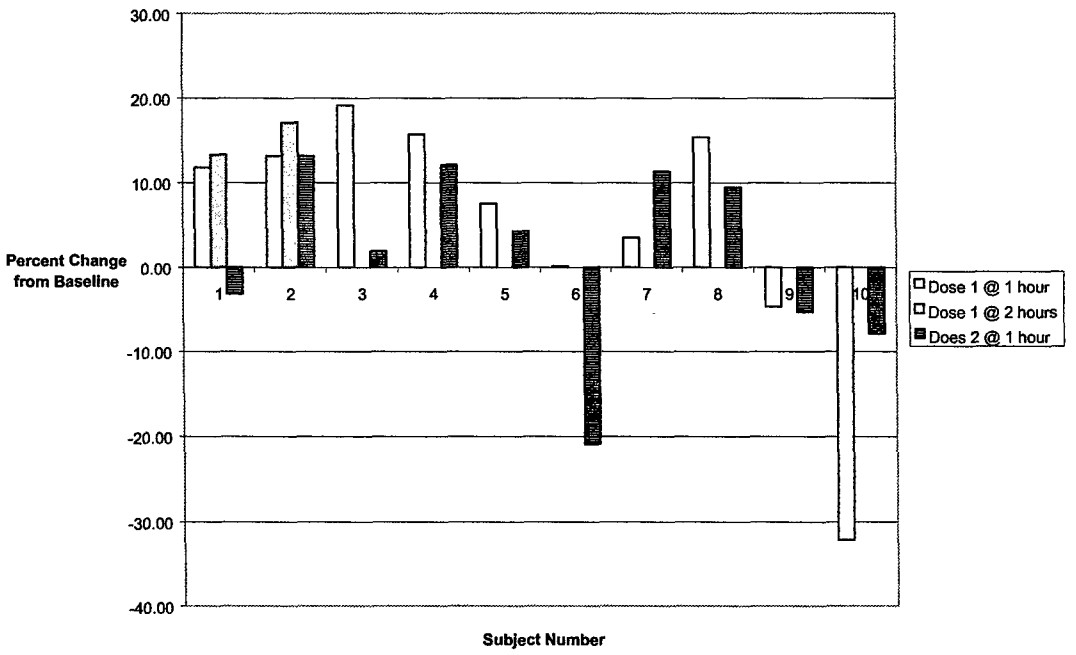


**Figure 9.** Percentage Change from Baseline in Finger Tapping with the Dominant Hand.

Table 10. Subject Means: Dominant Hand Finger Tapping

| | Averages mean ± SD |
|---|---|
| Baseline (n = 10) | 263.4 ± 18.0 |
| 1 hour after first dose (n = 10) | 270.7 ± 13.8 |
| 2 hours after first dose (n = 2) | 229.8 ± 34.3 |
| 1 hour after second dose (n = 10) | 263.1 ± 12.9 |

Nondominant Hand Finger Tapping

Most subjects showed at least 10% improvement in their baselines throughout the Nondominant Hand Finger Tapping test (Figure 9 ). Two subjects' performances decreased about 3%; another subject's, about 7%; and one subject had mixed results: one improvement and two slight decreases. The mean performances for nondominant hand finger tapping show that melatonin had little effect (Table 11).

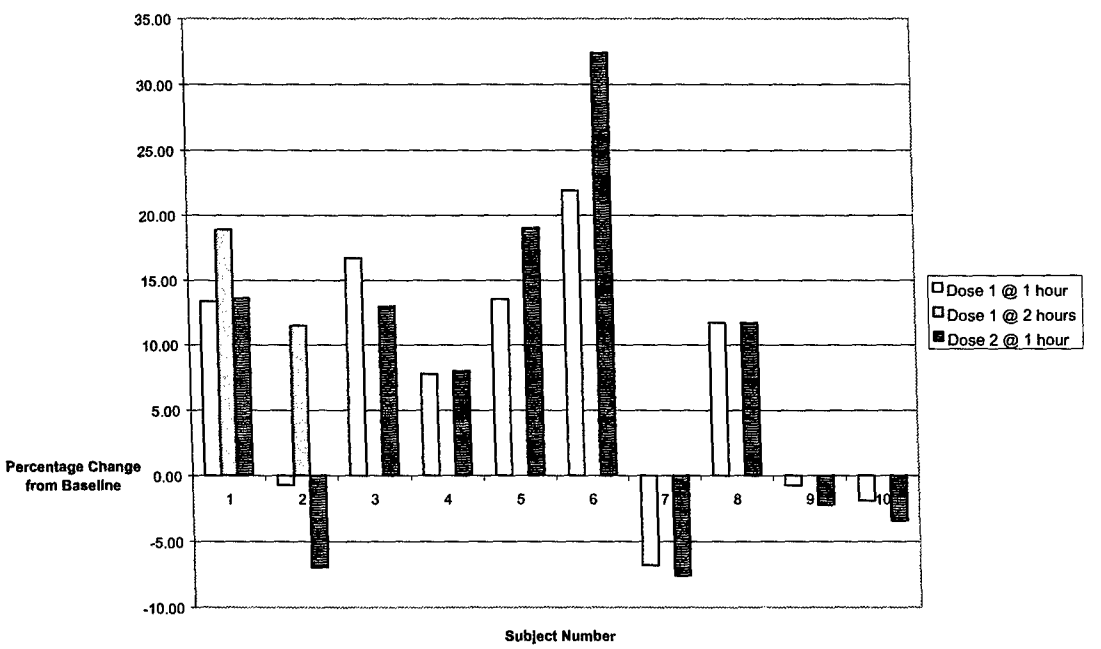


**Figure 10.** Percentage Change from Baseline in Finger Tapping with the Nondominant Hand.

Table 11. Subject Means: Nondominant Hand Finger Tapping

| | Averages mean ± SD |
|---|---|
| Baseline (n = 10) | 222.5 ± 32.1 |
| 1 hour after first dose (n = 10) | 237.0 ± 22.3 |
| 2 hours after first dose (n = 2) | 234.5 ± 2.1 |
| 1 hour after second dose (n = 10) | 237.0 ± 23.2 |

Sleepiness Scales 1 and 2

The sleepiness scales present, on the Table 1 scale of 1–7 (with 1 being wide awake), how frequently the subjects gave a particular response to characterize how tired they felt (Figures 11 and 12). Their responses are assessed with a relative scale that should afford some common, standard basis among subjects. The two tests are identical, except that the first was taken before each ANAM test was administered and the second after the ANAM testing was completed.

The baseline test cannot be compared to the data at two hours after the first dose, because only six subjects responded. The graph of data from Sleepiness Scale 1 shows that the baseline responses appear to be identical to the results one hour after the first dose of melatonin. The deviation occurs one hour after the second dose, when the responses of some subjects vary from their baselines. In Sleepiness Scale 2 (Figure 12), subjects indicate that they become more tired as time increases. This increased fatigue may indicate that the ANAM test caused them to become sleepy, but, in one notable exception, a subject had a baseline of 5 and his sleepiness scores decreased (he became more alert) throughout the study.

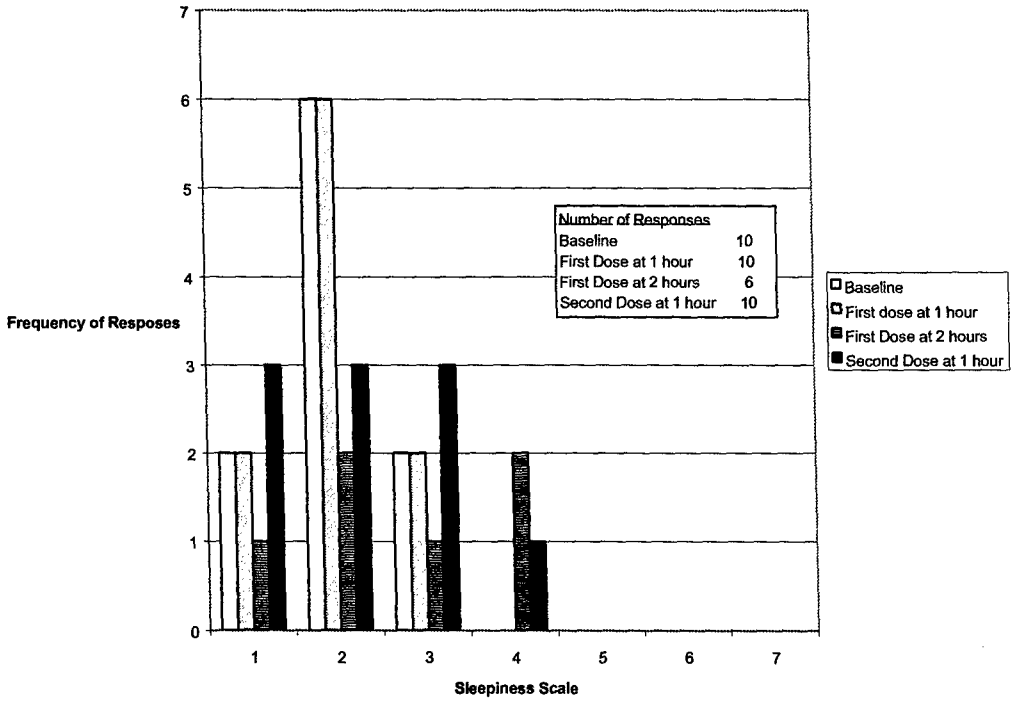


**Figure 11.** Stanford Sleepiness Scale 1.

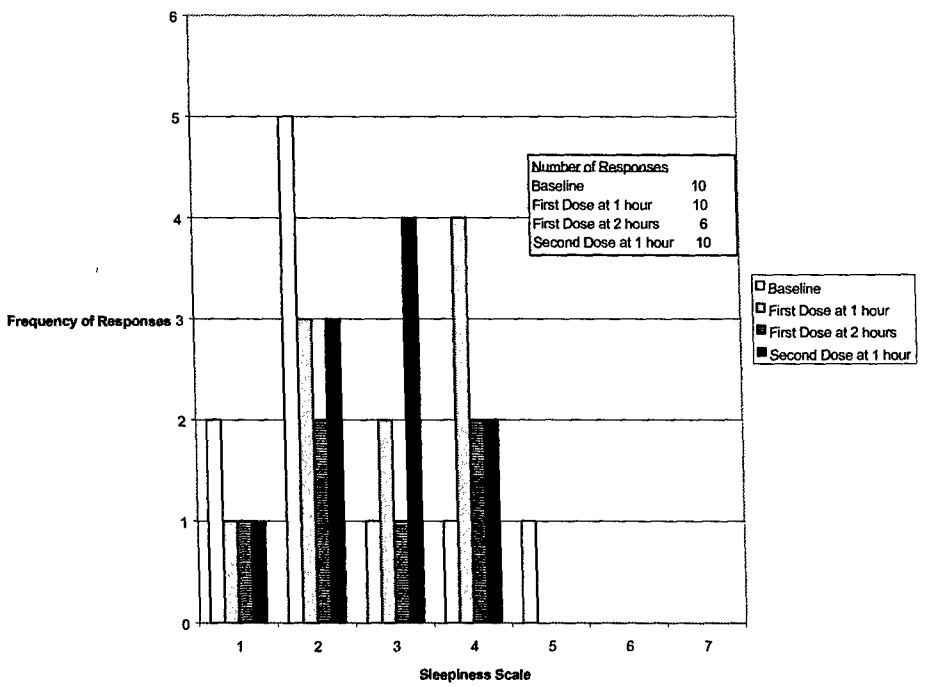


**Figure 12.** Stanford Sleepiness Scale 2.

## DISCUSSION

Overall, a total of 300 mg of pure melatonin ingested during the day by 10 physically fit adult subjects had no deleterious effect on an assortment of neuropsychological and physical measurements. The only trend noted over time and total dosage was an improvement in performance on Matching to Sample tests. That unexpected result was in some ways analogous to the reduction in "errors of commission" noted in 1984 tests of human performance after melatonin ingestion.[19] However, since this was a dosing study and all subjects received the same melatonin treatment, we cannot tell whether the improvement in Matching to Sample performance was due to the melatonin exposure or to some learning effect.

Hidden among the fairly consistent average values over time and dose were a few individual results from subjects who experienced relatively large deviations from baseline. These results might indicate either normal random variability or significant individual variability in both melatonin absorption and effect. In previous work, individual peak serum melatonin levels varied in healthy young subjects by a factor of 25 to 28 times,[20,21] and in one case as much as 300 times.[22] These serum-level variations occurred at both low doses (3 mg) and high doses (240 mg).

Melatonin has a relatively short half-life, and time determines the magnitude of its biological effects. In the 1984 Waldhauser study[20] of the bioavailability of orally administered melatonin (80–240 mg) in humans, peak serum melatonin levels were reached in 1 to 2.5 hr (absorption half-life of 0.4 hr), and remained constant for approximately 1.5 hr. Our ANAM measurements, made at one hour and sometimes two hours after the melatonin was administered, fell within the period of peak bioavailability.

Even though the first ANAM test was timed to occur at more than two absorption half-lives for melatonin,[20] in some subjects the one-hour test might have been taken before peak serum melatonin levels were reached. Nevertheless, the data from Waldhauser et al[20] suggests that following a 150 mg melatonin dose, serum levels one hour after dosing would have been far above those levels reported to cause sedation.[11,19]

### The Dopamine Connection

To put these dosing study results into perspective, some similarities between melatonin and dopamine pharmacokinetics are instructive. Although melatonin pharmacokinetics is less well studied than that of dopamine, we should be aware that, in general, both neurotransmitters (dopamine and melatonin) may vary in their physiological and pharmacological effects in a dose-dependent fashion, regardless of whether they are endogenous or administered exogenously. For example, dopamine has a concentration-dependent activation of various neurotransmitter receptor sites. Dopamine is an agonist of $\alpha$, $\beta$, and dopaminergic receptors with varying hemodynamic effects depending on dose.[23]

Plasma levels of dopamine in different individuals can also vary dramatically. For instance, one study using a homogeneous population of healthy male subjects and

weight-based dosing found a 10- to 75-fold intersubject variability in plasma dopamine concentrations.[23,24]

In addition, dopamine and melatonin share some teleological similarities. Dopamine is a catecholamine neurotransmitter derived from the amino acid tyrosine. Melatonin is a neurotransmitter derived from serotonin (5-hydroxytryptamine [5-HT]), which in turn is derived from the amino acid tryptophan. Melatonin acts by inhibiting the synthesis and secretion of dopamine and other neurotransmitters. Like dopamine, it acts on at least two different receptors, Mel1a and Mel1b (sometimes called ML-1 and ML-2, or MT1 and MT2, respectively).[25–28]

The following is a quote regarding dopamine pharmacokinetics from MacGregor et al (2000):

> Dopamine is an endogenous catecholamine that regulates cardiac, vascular, and endocrine function. Dopamine is also used clinically to support organ function and to modulate hemodynamics in critically ill patients. Conventional teaching states that when exogenous dopamine is infused at low doses (between 0.5 and 3.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) the predominant effects are stimulation of dopaminergic receptors with resultant increases in splanchnic (including renal) blood flow, diuresis, and natriuresis. At higher doses (>3.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$), β-adrenergic stimulation predominates, increasing cardiac inotropy and chronotropy. Doses >7.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ result in predominant α-adrenergic stimulation, resulting in peripheral and splanchnic vasoconstriction. Despite this conventional wisdom, clinical experience demonstrates that there is considerable interpatient variability in response to dopamine infusion, even when administered at identical rates.[24]

If the action of melatonin is in any way analogous to that of dopamine, then we should not be surprised that human subjects often report no drowsiness as a result of 150–300 mg melatonin doses, whereas they usually report 1–3 mg doses to be soporific. Likewise, because plasma levels of melatonin can vary widely, it is not surprising that some of our test subjects became somnolent following high melatonin doses administered orally, while other subjects did not.

## Melatonin Analogs

Melatonin analogs improve upon the potency and half-life of melatonin and also have the potential, so far unproven, of reducing soporific effects. For instance, indole-3-propionic acid (IPA) is a deamination product of tryptophan, with a structure similar to that of melatonin. Like melatonin, IPA is naturally present in biological fluids and is an effective free radical scavenger. Due to its antioxidant potency, IPA has been found to inhibit formation of beta-amyloid fibrils in brains,[29] a debilitating characteristic of Alzheimer's disease, and to protect against a variety of oxidotoxins.[30, 31] Along with melatonin, IPA protects completely against carcinogen-induced DNA damage in hamster kidneys.[32] IPA is now a pharmaceutical-grade antioxidant patented for treatment of Alzheimer's disease[33] and is known commercially as Oxigon. IPA is in FDA

Phase 1 trials in the U.S. and Phase 2 trials in Europe. Whether IPA has any soporific side effects is yet unknown.

Melatonin has a very short half-life, approximately 25 minutes in man.[20] IPA has a half-life of more than three hours, but it penetrates the blood-brain barrier more slowly than melatonin does. The patent holders for IPA currently have a patent submitted for an antioxidant that has an even longer half-life than that of IPA and which penetrates the blood-brain barrier as well as melatonin does.

## CONCLUSIONS

Virtually every neuropsychological subtest had at least one person experience some deficit. However, statistically melatonin had no deleterious effects on mental performance and alertness as measured by ANAM, or on physical performance measured by grip strength, finger tapping speed, and the Harvard Step Test.

Unlike the effects of small doses of melatonin reported in the literature, the large doses applied in this study did not trigger any adverse reactions, but they did transiently reduce about half the subjects' feelings of overall alertness. One possible explanation for these seemingly contradictory results is a paradoxical dose-response effect in which higher doses manage to eliminate the drowsiness reported in studies employing much lower doses. Although this hypothesis is merely conjecture, the pharmacological effects of other neurotransmitters, such as dopamine, do behave in a dose-dependent biphasic manner, thereby lending credence to this hypothesis.

## RECOMMENDATIONS

While results from this study do not categorically qualify melatonin for use during military operations, they suggest that the large doses of melatonin we employed are relatively innocuous, at least as evidenced by psychometric testing. This finding agrees with the growing literature on melatonin. Concern about cognitive impairment should not limit research on melatonin as a candidate pharmacological agent for minimizing oxygen-induced pulmonary damage.

## REFERENCES


1. Naval Sea Systems Command, *Task Number 99-04c: Antioxidants as Protectants against $O_2$ Toxicity*, June 1999.

2. Commander, Naval Sea Systems Command, *U. S. Navy Diving Manual, Revision 4*, Publication SS521-AG-PRO-010 (Arlington, VA: NAVSEA, 1999), Vol. 1.

3. M. I. Pablos, R. J. Reiter, J. I. Chuang, G. G. Ortiz, J. M. Guerrero, E. Sewerynek, M. T. Agapito, D. Melchiorri, R. Lawrence, and S. M. Deneke, "Acutely Administered Melatonin Reduces Oxidative Damage in Lung and Brain Induced by Hyperbaric Oxygen," *J. Appl. Physiol.*, Vol. 83, No. 2 (1997), pp. 354–358.

4. M. Hara, M. Ligo, R. Ohtani-Kaneko, N. Nakamura, T Suzuki, R. J. Reiter, and K. Hirata, "Administration of Melatonin and Related Indoles Prevents Exercise-Induced Cellular Oxidative Changes in Rats," *Biol. Signals*, Vol. 6, No. 2 (1997), pp. 90–100.

5. J. M. Jacobson, J. R. Michael, M. H. Jafri Jr, and G. H. Gurtner, "Antioxidants and Antioxidant Enzymes Protect against Pulmonary Oxygen Toxicity in the Rabbit," *J. Appl. Physiol.*, Vol. 68, No. 3 (1990), pp. 1252–1259.

6. R. J. Reiter, "Functional Aspects of the Pineal Hormone Melatonin in Combating Cell and Tissue Damage Induced by Free Radicals," *Eur. J. Endocrinol.*, Vol. 134, No. 4, (1996), pp. 412–420.

7. J. Barchus, F. daCosta, and S. Spector, "Acute Pharmacology of Melatonin," *Nature*, Vol. 214 (1967), pp. 919–920.

8. E. J. Pappert, C. C. Tangney, C. G. Goetz, Z. D. Ling, G. T. Stebbins, and P. M. Carvey, "Alpha-tocopherol in the Ventricular Cerebrospinal Fluid of Parkinson's Disease Patients: Dose-Response Study and Correlations with Plasma Levels," *Neurology*, Vol. 47, No. 4 (1996), pp. 1037–1042.

9. B. C. G. Voordouw, R. Euser, R. E. Verdonk, B. T. Alberta, F. H. de Jong, A. C. Drogendijk, B. C. Fauser, and M. Cohen, "Melatonin and Melatonin-Progestin Combinations Alter Pituitary-Ovarian Function in Women and Can Inhibit Ovulation," *J. Clin. Endocrinol. Metab.*, Vol. 74, No. 1 (1992), pp. 108–117.

10. R. Valcavi, C. Dieguez, C. Azzarito, C. A. Edwards, C. Dotti, M. D. Page, I. Portioli, and M. F. Scanlon, "Effect of Oral Administration of Melatonin on GH Responses to GRF I-44 in Normal Subjects," *Clin. Endocrinol.*, Vol. 26, No. 4 (1987), pp. 453–458.

11. A. B. Dollins, H. J. Lynch, R. J. Wurtman, M. H. Deng, K. U. Kischka, R. E. Gleason, and H. R. Lieberman, "Effect of Pharmacological Daytime Doses of Melatonin on Human Mood and Performance," *Psychopharmacology*, Vol. 112, No. 4 (1993), pp. 490–496.

12. R. K. Heaton, I. Grant, and C. G. Matthews, *Comprehensive Norms for an Expanded Halstead-Reitan Battery: Demographic Corrections, Research Findings, and Clinical Applications* (Odessa, FL: Psychological Assessment Resources, 1991).

13. L. J. Crepeau and J. R. Clarke, *Evaluating the Effects of High-Dose Melatonin on Mental and Somatic Status*, NEDU TP 99-32 (Limited Distribution), Navy Experimental Diving Unit, Nov 1999.

14. D. Reeves, R. Kane, K. Winter, and A. Goldstone, *Tester's Workbench Automated Neuropsychological Assessment Metrics (ANAM): Clinical and Neurotoxicology Subsets. User's Manual and Documentation* (Washington, DC: Department of the Army, Office of Military Performance Assessment Technology, 1993).

15. D. Reeves, T. Elsmore, K. Winter, R. Kane, and J. Bleiberg, *ANAM 2000 (Beta 1.0) User's Manual, NCRF/NRH Special Report 98-01* (Washington, DC: The National Rehabilitation Hospital [NRH], 1998).

16. D. L. Reeves, K. P. Winter, S. J. LaCour, K. M. Raynsford, K. Vogel, and J. D. Grissett, *The UTC-PAB/AGARD STRES Battery: User's Manual and System Documentation*, NAMRL Special Report 91-3, Naval Aerospace Medical Research Laboratory, 1991.

17. D. R. Thorne, S. G. Genser, H. C. Sing, and F. W. Hegge, "The Walter Reed Performance Assessment Battery," *Neurobehavioral Toxicology and Teratology*, Vol. 7 (1985), pp. 415–418.

18. D. Hyde, J. R. Thomas, J. Schrot, and W. F. Taylor, *Quantification of Special Operations Mission-Related Performance: Operational Evaluation of Physical Measures*, Naval Medical Research Institute Report 97-01, 1997.

19. H. R. Lieberman, F. Waldhauser, G. Garfield, H. J. Lynch, and R. J. Wurtman, "Effects of Melatonin on Human Mood and Performance," *Brain Res.*, Vol. 323, No. 2 (1984), pp. 201–207.

20. F. Waldhauser, M. Waldhauser, H. R. Lieberman, M. H Deng, H. J. Lynch, and R. J. Wurtman, "Bioavailability of Oral Melatonin in Humans," *Neuroendocrinology*, Vol. 39, No. 4 (1984), pp. 307–313.

21. J. Kovacs, W. Brodner, V. Kirchlechner, T. Arif, and F. Waldhauser, "Measurement of Urinary Melatonin: A Useful Tool for Monitoring Serum Melatonin after Its Oral Administration," *J Clin Endocrinol Metab*, Vol. 85, No. 2 (2000), pp. 666–670.

22. F. Waldhauser, B. Saletu, and I. Trinchard-Lugan, "Sleep Laboratory Investigations on Hypnotic Properties of Melatonin," *Psychopharmacology* (Berlin), Vol. 100, No. 2 (1990), pp. 222–226.

23. B. B. Hoffman and R. J. Lefkowitz, "Catecholamines and Sympathomimetic Drugs," in A. G. Gilman, T. W. Rall, A. S. Nies, and P. Taylor, eds., *Goodman and Gilman's*

*The Pharmacological Basis of Therapeutics*, 8th ed. (New York: Pergamon Press, 1990), p. 201.

24. D. A. MacGregor, T. E. Smith, R. C. Prielipp, J. F. Butterworth, R. L. James, and P. E. Scuderi, "Pharmacokinetics of Dopamine in Healthy Male Subjects," *Anesthesiology*, Vol. 92, No. 2 (2000), pp. 338–346.

25. M. L. Dubocovich, "Pharmacology and Function of Melatonin Receptors," *FASEB Journal*, Vol. 2 (1988), pp. 2765–2773.

26. L. Petit, I. Lacroix, P. de Coppet, A. D. Strosberg, and R. Jockers, "Differential Signaling of Human Mel1a and Mel1b Melatonin Receptors through the Cyclic Guanosine 3'-5'-Monophosphate Pathway," *Biochem. Pharmacol.*, Vol. 58, No. 4 (1999), pp. 633–639.

27. W. M. Al-Ghoul, M. D. Herman, and M. L. Dubocovich, "Melatonin Receptor Subtype Expression in Human Cerebellum," *Neuroreport*, Vol. 9 (1998), pp. 4063–4068.

28. C. Von Gall, J. H. Stehle, and D. R. Weaver, "Mammalian Melatonin Receptors: Molecular Biology and Signal Transduction," *J. Cell Tissue Res.*, Vol. 309 (2002), pp. 151–162.

29. P. E. Bendheim, B. Poeggeler, E. Neria, V. Ziv, M. A. Pappolla, and D. G. Chain, "Development of Indole-3-Propionic Acid (OXIGON) for Alzheimer's Disease," *J. Mol. Neurosci.*, Vol. 19, No. 1–2 (2002), pp. 213–217.

30. M. Karbownik, E. Gitto, A. Lewinski, and R. J. Reiter, "Relative Efficacies of Indole Antioxidants in Reducing Autoxidation and Iron-Induced Lipid Peroxidation in Hamster Testes," *J. Cell Biochem.*, Vol. 81, No. 4 (2001), pp. 693–699.

31. M. Karbownik, R. J. Reiter, J. J. Garcia, J. Cabrera, S. Burkhardt, C. Osuna, and A. Lewinski, "Indole-3-Propionic Acid, a Melatonin-Related Molecule, Protects Hepatic Microsomal Membranes from Iron-Induced Oxidative Damage: Relevance to Cancer Reduction," *J. Cell Biochem.*, Vol. 81, No. 3 (2001), pp. 507–513.

32. M. Karbownik, R. J. Reiter, J. Cabrera, and J. J. Garcia, "Comparison of the Protective Effect of Melatonin with Other Antioxidants in the Hamster Kidney Model of Estradiol-Induced DNA Damage," *Mutat. Res.*, Vol. 474, No. 1–2 (2002), pp. 87–92.

33. M. A. Pappolla, B. Frangione, J. Ghiso, and B. Poeggeler, *Uses for Indole-3-Propionic Acids and Salts and Esters Thereof*, U.S. Patent no. 6,395,768, May 28, 2002.

## APPENDIX A

MELATONIN DOSING POSTEXPOSURE QUESTIONNAIRE, WITH DIVER RESPONSES INSERTED BELOW EACH QUESTION

(1) Did you experience any unusual sensations after taking the melatonin? Please describe any specific feelings, symptoms, difficulty solving problems, etc.

a. The first 40 minutes after taking melatonin the first time, I felt a little lightheaded, much the same as I would if I guzzled a can of Mountain Dew soda.

b. I had a slight "glazed" feeling, particularly during the first hour after taking the melatonin. This effect seemed to be blunted after the second administration.

c. Yes. After the second dose I felt more sleepy than usual. I had difficulty staying awake while working at my desk. I felt completely normal after the first dose.

d. None.

e. No.

f. After first dose and before physical activity, very "mellow, sleepy." Slight difficulty in concentrating and keeping focus (not visual) on tasks.

g. No . . . but I exercised.

h. Fatigue, slight irritability, a little "dopey" feeling all after first dose. I didn't feel like my problem solving skills were impaired, I was just irritated to do the entire ANAM [Performance Assessment Battery]. Somewhat distracted. After the second dose, I was impaired. Depth perception was a little off, slight vertigo, very irritable.

i. No, it seems to take forever to do small tasks but then when looking at the clock it really wasn't that long.

j.* Very drowsy, irritable, difficulty keeping eyes open at my desk.

(2) Comment on your ability to maintain concentration. Easy? Difficult? Please elaborate on any specific cognitive tasks that were particularly difficult.

a. No impairments to my cognitive abilities were noted.

b. My ability to concentrate did not appear materially affected, although I felt such a sense of ease that I wasn't as diligent on some tasks as I would have been.

c. It was difficult only after the 2nd dose. I had difficulty staying awake while writing a report.

d. No more problems than usual. Felt mellow — took edge off stress.

e. Normal.

f. After first dose and before physical activity, very "mellow, sleepy." Slight difficulty in concentrating and keeping focus (not visual) on tasks.

g. I felt fine.

h. Concentration was not good at all. The [matching to sample] test was particularly difficult for me. Again, I felt irritable over all.

i. It was fairly easy to maintain concentration.

j.* Normal to slightly difficult, due to drowsiness.

(3) How would you feel about administering the same dose of melatonin to other people? Please elaborate on areas where you would have concerns about administering melatonin.

a. I would not hesitate to administer it or take it myself again. You might want to investigate if a different type of diluent (i.e., orange juice) would help hide the slight taste of the melatonin. Perhaps V8 juice? Proceed to Phase II.

b. I don't think I'd have any problem giving it to people under most circumstances. I would ensure that someone with a very demanding job (e.g., fighter pilot) felt comfortable flying after taking melatonin. But for most tasks, I wouldn't have a problem giving it to people.

c. I think that if they can nap, or are active, it won't matter that they may feel sleepy. I have no concerns unless their lives depend on their diligence, concentration, or cognition while sedentary.

d. Okay for others.

e. No concerns.

f. Concerned at this dose when operators have to make "split second," especially tactical decisions.

g. I understand it was a very large dose?

h. Dose is too high. If that amount was administered, the person would need to go home or get SIQ'd [sent home as Sick In Quarters]. Also, using alcohol as the vehicle to administer may be a concern for recovering alcoholics.

i. No problem. If they were going to perform brain surgery or even surgery on me I'd probably say no.

j.* No problem unless they were driving a vehicle.

(4) How well could you move your hands and manipulate objects (fine motor control)? How well could you walk, lift, etc. (gross motor control)? Please elaborate on any specific activities that you found particularly difficult, due to perceived motor inhibition.

a. I noted no impairments to either my fine/gross motor controls.

b. Gross and fine motor control remained unaffected.

c. I felt no motor changes.

d. Had no difficulty.

e. Normal.

f. Coordination and motor OK.

g. I had no problems at all. I think that in a situation where I couldn't have exercised it would have had a different effect on me.

h. The "step-up" was difficult after the first dose — felt a little lazy. After the second dose, it felt like a real effort.

i. None.

j.* Fine.

(5) What is your overall impression of this agent's influence on your mental abilities and motor performance levels (please include all factors, such as how it affects your thinking, feeling, somatic sensations)?

a. Other than the slight "buzz" the first 40 minutes, I noted no other symptoms. If anything, it made me feel more awake rather than somnolent.

b. Again, except for not caring as much about my performance, I don't believe that melatonin reduced my capacity to perform in many different areas. Indeed, it may be that the sense of peace I felt would provide some level of disinhibition — without the concomitant reduction in motor reaction speed or accuracy — that could prove valuable in some situations (e.g., where it is harmful to hesitate).

c. I had not emotional peaks or troughs that I noticed. I felt fine physically, except for increased sleepiness after taking the 2nd dose.

d. Mental / motor abilities not affected.

e. No effect.

f. Would not take before critical decision task, a little too "relaxed, mellow."

g. Same comment [as number (4)]

h. I felt irritable, drowsy, a little depressed.

i. It felt like you had been out partying Friday and Saturday, then have to get up and go to church.

j.* Little to no influence on mental / motor.

(6) Any suggestions about using this agent in an operational setting? Please provide additional comments on the back of this form.

a. Take at least one hour prior to commencing operations — I would not want to have to make life or death decisions with a melatonin-induced "buzz!"

b. In most operational settings I don't believe this agent would negatively affect performance levels.

c. I felt fine physically, except for increased sleepiness after taking the 2nd dose.

d. I would give over several days before mission — additive effect and habituation.

e. None.

f. Personally, would not use, e.g., SDV operation, especially for pilot and navigator.

g. If it was planned to be used by operational units at sea they wouldn't be afforded the opportunity to exercise. Maybe it should be tested in the operational scenario?

h. Not a good idea. I would be very concerned about the person's ability to perform. He would probably need to be SIQ'd after dosing.

i. None.

j.* Need to somehow counter drowsy effects.

*Subject had suffered from flu-like symptoms two days before, and the night after, being tested.

## APPENDIX B. Raw Data from ANAM Tests

| Reaction Time | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 261 | 303.24 | 300.28 | 300 |
| 2 | 258.2 | 287.32 | 257.64 | 288 |
| 3 | 286.12 | 294.96 | 243 | 275.48 |
| 4 | 236.04 | 234.22 | | 235.33 |
| 5 | 244.88 | 229.84 | 225.08 | 226.68 |
| 6 | 262.12 | 253.04 | | 266.96 |
| 7 | 267.58 | 295.16 | | 266.44 |
| 8 | 249.96 | 244.21 | | 279.44 |
| 9 | 229 | 229 | | 237 |
| 10 | 225 | 237 | | 231 |

| Matching (Thruput) | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 37.57 | 35.56 | 35.53 | 39.03 |
| 2 | 34.73 | 47.61 | 47.91 | 43.64 |
| 3 | 25.07 | 29.92 | 38.04 | 33.06 |
| 4 | 31.76 | 48.85 | | 93.89 |
| 5 | 56.86 | 37.16 | 53.85 | 57.18 |
| 6 | 39.09 | 41.52 | | 40.21 |
| 7 | 32.07 | 43.57 | | 45.34 |
| 8 | 51.61 | 57.85 | | 54.65 |
| 9 | 25.47 | 50.42 | | 37.86 |
| 10 | 65.5 | 75.62 | | 85.49 |

| Running Memory (Thruput) | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 97.03 | 95.96 | 103.1 | 114.7 |
| 2 | 51.08 | 94.86 | 109.13 | 100.51 |
| 3 | 126.9 | 107.12 | 126.38 | 120.37 |
| 4 | 138 | 146.51 | | 157.94 |
| 5 | 133.38 | 149.77 | 150.71 | 152.99 |
| 6 | 149.9 | 146.65 | | 137.51 |
| 7 | 124.25 | 131.04 | | 138.35 |
| 8 | 123.18 | 127.74 | | 120.63 |
| 9 | 62.62 | 107.31 | | 118.46 |
| 10 | 143.59 | 140.01 | | 136.31 |

| Math Processing (Thruput) | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 31.14 | 18.42 | 17.17 | 15.73 |
| 2 | 31.83 | 32.09 | 36.64 | 33.83 |
| 3 | 34.98 | 29.61 | 38.75 | 37.77 |
| 4 | 32.94 | 44.62 | | 32.49 |
| 5 | 29.67 | 30.52 | 40.88 | 33.67 |
| 6 | 34.96 | 38.22 | | 42.77 |
| 7 | 45.59 | 38.99 | | 44.28 |
| 8 | 37.55 | 36.42 | | 42.31 |
| 9 | 27.8 | 28.82 | | 24.67 |
| 10 | 44.32 | 26.03 | | 32.68 |

| Sleepiness 1 | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 2 | 1 | 1 | 2 |
| 2 | 2 | 2 | 3 | 3 |
| 3 | 2 | 2 | 2 | 2 |
| 4 | 2 | 1 | | 1 |
| 5 | 3 | 2 | 2 | 3 |
| 6 | 1 | 2 | | 1 |
| 7 | 2 | 2 | | 2 |
| 8 | 1 | 2 | | 1 |
| 9 | 2 | 3 | 4 | 4 |
| 10 | 3 | 3 | 4 | 3 |

| Sleepiness 2 | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 2 | 1 | 1 | 3 |
| 2 | 5 | 3 | 3 | 3 |
| 3 | 2 | 4 | 2 | 2 |
| 4 | 2 | 4 | | 2 |
| 5 | 3 | 4 | 2 | 4 |
| 6 | 1 | 2 | | 2 |
| 7 | 2 | 2 | | 3 |
| 8 | 1 | 2 | | 1 |
| 9 | 2 | 3 | 4 | 4 |
| 10 | 4 | 4 | 4 | 3 |

| Dominant Hand Grip Strength | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 26 | 22 | 22 | 25 |
| 2 | 49 | 45.5 | 48 | 48 |
| 3 | 41 | 41 | | 49 |
| 4 | 46 | 48 | | 47 |
| 5 | 30 | 32 | | 32 |
| 6 | 39 | 40 | | 44 |
| 7 | 54 | 64 | | 62 |
| 8 | 62 | 62 | | 60 |
| 9 | 58 | 66 | | 58 |
| 10 | 54 | 50 | | 52 |

| Nondominant Hand Grip Strength | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 23 | 15 | 16 | 22 |
| 2 | 39 | 37 | 39 | 39 |
| 3 | 44 | 36 | | 49 |
| 4 | 34 | 36 | | 34 |
| 5 | 27 | 27 | | 27 |
| 6 | 32 | 34 | | 34.5 |
| 7 | 48 | 46 | | 58 |
| 8 | 64 | 64 | | 64 |
| 9 | 55 | 58 | | 54 |
| 10 | 50 | 48 | | 46 |

| Dominant Hand Finger Tapping | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 233 | 260.5 | 264 | 225.5 |
| 2 | 167 | 189 | 195.5 | 189 |
| 3 | 209 | 249 | | 213 |
| 4 | 235.5 | 272.5 | | 264 |
| 5 | 292 | 314 | | 304.5 |
| 6 | 346.5 | 347 | | 274 |
| 7 | 256 | 265 | | 285 |
| 8 | 254 | 293 | | 278 |
| 9 | 299 | 285 | | 283 |
| 10 | 342 | 232 | | 315 |

| Nondominant Hand Finger Tapping | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 198.5 | 225 | 236 | 225.5 |
| 2 | 209 | 207.5 | 233 | 194.5 |
| 3 | 200.5 | 234 | | 226.5 |
| 4 | 225 | 242.5 | | 243 |
| 5 | 229 | 260 | | 272.5 |
| 6 | 162 | 197.5 | | 214.5 |
| 7 | 250 | 233 | | 231 |
| 8 | 222 | 248 | | 248 |
| 9 | 267 | 265 | | 261 |
| 10 | 262 | 257 | | 253 |

| Harvard Step Test | | | | |
|---|---|---|---|---|
| Subject Number | Baseline | 1 hour after first dose | 2 hours after first dose | 1 hour after second dose |
| 1 | 32 | 40 | 36 | 38 |
| 2 | 53 | 40 | 47 | 43 |
| 3 | 38 | 41 | | 42 |
| 4 | 32 | 36 | | 32 |
| 5 | 42 | 44 | | 47 |
| 6 | 41 | 45 | | 46 |
| 7 | 36 | 36 | | 36 |
| 8 | 32 | 37 | | 38 |
| 9 | 29 | 33 | | 34 |
| 10 | 45 | 45 | | |